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PHARMACOLOGY

Microdialysis Study of Aztreonam-Avibactam Distribution in Peritoneal Fluid and Muscle of Rats with or without Experimental Peritonitis

Alexia Chauzy,[a] Isabelle Lamarche,[a] Christophe Adier,[a,b] William Couet,[a,b] Sandrine Marchand[a,b]

[a]INSERM, U1070, UFR de Médecine Pharmacie, Université de Poitiers, TSA 51106, Poitiers, France
[b]Laboratoire de Toxicologie-Pharmacocinétique, CHU of Poitiers, Poitiers, France

ABSTRACT The purpose of this study was to investigate aztreonam (ATM) and avibactam (AVI) distribution in intraperitoneal fluid and muscle interstitial fluid by microdialysis in rats, with or without peritonitis, and to compare the unbound concentrations in tissue with the unbound concentrations in blood. Microdialysis probes were inserted into the jugular veins, hind leg muscles, and peritoneal cavities of control rats ($n = 5$) and rats with intra-abdominal sepsis ($n = 9$) induced by cecal ligation and punctures. ATM and AVI probe recoveries in each medium were determined for both molecules in each rat by retrodialysis by drug. ATM-AVI combination was administered as an intravenous bolus at a dose of 100-25 mg · kg^{-1}. Microdialysis samples were collected over 120 min, and ATM-AVI concentrations were determined by liquid chromatography-tandem mass spectrometry. Noncompartmental pharmacokinetic analysis was conducted and nonparametric tests were used for statistical comparisons between groups (infected versus control) and medium. ATM and AVI distribution in intraperitoneal fluid and muscle was rapid and complete both in control rats and in rats with peritonitis, and the concentration profiles in blood, intraperitoneal fluid, and muscle were virtually superimposed, in control and infected animals, both for ATM and AVI. No statistically significant difference was observed between unbound tissue extracellular fluid and systemic areas under the curve for both molecules in control and infected animals. In the present study, intraperitoneal infection induced by cecal ligation and puncture had no apparent effect on ATM and AVI pharmacokinetics in rats.

KEYWORDS microdialysis, aztreonam-avibactam, intraperitoneal infection, pharmacokinetics

Intra-abdominal infections are common and represent the second major reason (after lung infections) for septic shock in intensive care units (1). While surgery is the first-line treatment in the management of peritonitis, appropriate antimicrobial therapy, including the administration of a suitable molecule at an optimized dosing regimen, is also essential to prevent recurrent intra-abdominal infection, reduce surgical wound complications, and control bacteremia (2, 3).

Pharmacokinetic studies of antibiotics most often rely on plasma data. However, since most infections occur in tissue extracellular fluid (ECF), unbound antibiotic concentrations in the interstitial fluid at the target site are more useful for predicting the antibacterial effect and optimal drug dosing regimens (4). Although in most tissues unbound drug concentrations at equilibrium should be equal in plasma and tissue ECF (5), some situations such as the presence of efflux transporters or a peripheral degradation in tissue can lead to a less unbound tissue ECF than plasma areas under the curve (AUC) (6). Microdialysis is a well-known technique that can be used for determination of unbound drug concentrations, including antibiotics in tissue interstitial fluid,

Received 8 June 2018 **Returned for modification** 1 July 2018 **Accepted** 13 July 2018

Accepted manuscript posted online 16 July 2018

Citation Chauzy A, Lamarche I, Adier C, Couet W, Marchand S. 2018. Microdialysis study of aztreonam-avibactam distribution in peritoneal fluid and muscle of rats with or without experimental peritonitis. Antimicrob Agents Chemother 62:e01228-18. https://doi.org/10.1128/AAC.01228-18.

Address correspondence to Sandrine Marchand, sandrine.marchand@univ-poitiers.fr.

and which rapidly gained popularity in pharmacokinetic and pharmacodynamic studies, both in experimental animals and in humans (7, 8).

Aztreonam-avibactam (ATM-AVI) is a novel β-lactam–β-lactamase inhibitor combination under development for the treatment of serious Gram-negative infections, including complicated intra-abdominal infections, proven or strongly suspected to be caused by multiple-drug resistant pathogens producing metallo-β-lactamases (9). ATM-AVI combination provides broad coverage against *Enterobacteriaceae* strains that produce broad-spectrum β-lactamases (10) and which have been commonly reported to cause peritonitis (3, 11).

The objective of this study was to investigate ATM and AVI distribution within peritoneal fluid (PF) and muscle interstitial fluid by microdialysis and to compare intraperitoneal unbound concentrations with unbound concentrations in blood in healthy rats and in rats with experimental peritonitis caused by cecal ligation and puncture.

RESULTS

Lactate and glucose concentrations in plasma. Lactate concentrations were significantly higher in rats with peritonitis than in control rats ($P < 0.05$), and were, respectively, equal to 4.30 ± 0.89 mmol · liter^{-1} and 2.42 ± 0.36 mmol · liter^{-1}. Inversely, glucose concentrations were significantly lower in rats with peritonitis (1.34 ± 0.17 g · liter^{-1}) than in control rats (1.85 ± 0.06 g · liter^{-1}) ($P < 0.05$).

Pharmacokinetic study. ATM and AVI *in vivo* recovery by loss ($RL_{in\ vivo}$) differed between rats for the same medium and between different media for the same rat, but there was no trend between control and infected rats. However, probe recoveries were generally lower for ATM than for AVI. Therefore, whatever the group, the relative $RL_{in\ vivo}$ of ATM varied between 11.2% ± 1.9% and 46.2% ± 0.9% in blood, between 14.4% ± 2.8% and 52.2% ± 1.1% in muscle, and between 17.3% ± 1.1% and 50.2% ± 5.8% in PF. AVI probe recoveries varied between 27.3% ± 1.6% and 66.8% ± 1.5% in blood, between 25.6% ± 1.5% and 53.5% ± 1.6% in muscle, and between 26.1% ± 0.4% and 69.6% ± 1.0% in PF.

(i) Control group. ATM and AVI blood, muscle, and PF concentrations measured in all rats included in the control group were used for the pharmacokinetic analysis. In these three media, the decay of unbound concentrations with time was monoexponential (Fig. 1b and 2b), and the concentration profiles were almost superimposed for ATM, as well as AVI (Fig. 1a and 2a). Unbound peak concentrations in muscle and PF were observed within the first 10-min collection interval and were not delayed compared to the unbound peak concentrations in blood. Pharmacokinetic parameter values obtained in the control group are presented in Table 1 for ATM and Table 2 for AVI. Half-lives and AUCs of ATM and AVI were not statistically different between blood, muscle, and PF, and the AUC ratios were close to 1 (Tables 1 and 2).

(ii) Peritonitis group. ATM and AVI concentrations measured in all animals were kept for the pharmacokinetic analysis, except for ATM muscle concentrations in one infected rat in which the ATM probe recovery in muscle was very low (<10%). The decay of unbound ATM and AVI concentrations in blood, muscle, and PF with time was still monoexponential (Fig. 1d and 2d), and the concentration-versus-time profiles in these three media were also virtually superimposed for both molecules with no delay when comparing peak concentrations in muscle and PF with peak concentrations in blood (Fig. 1c and 2c). Greater between-rat variability was observed in infected animals than in control rats (Fig. 1a and c and Fig. 2a and c). Pharmacokinetic parameters values obtained in the control and peritonitis groups (Table 1 for ATM and Table 2 for AVI) were not significantly different. Half-lives and AUCs were not statistically different between blood, muscle, and PF, with AUC ratios still being close to 1.

DISCUSSION

The main purpose of this study was to evaluate ATM and AVI distribution in PF and muscle in healthy and infected rats using microdialysis. Muscle was chosen to be a control since only simple bidirectional passive diffusion occurs in this tissue (12).

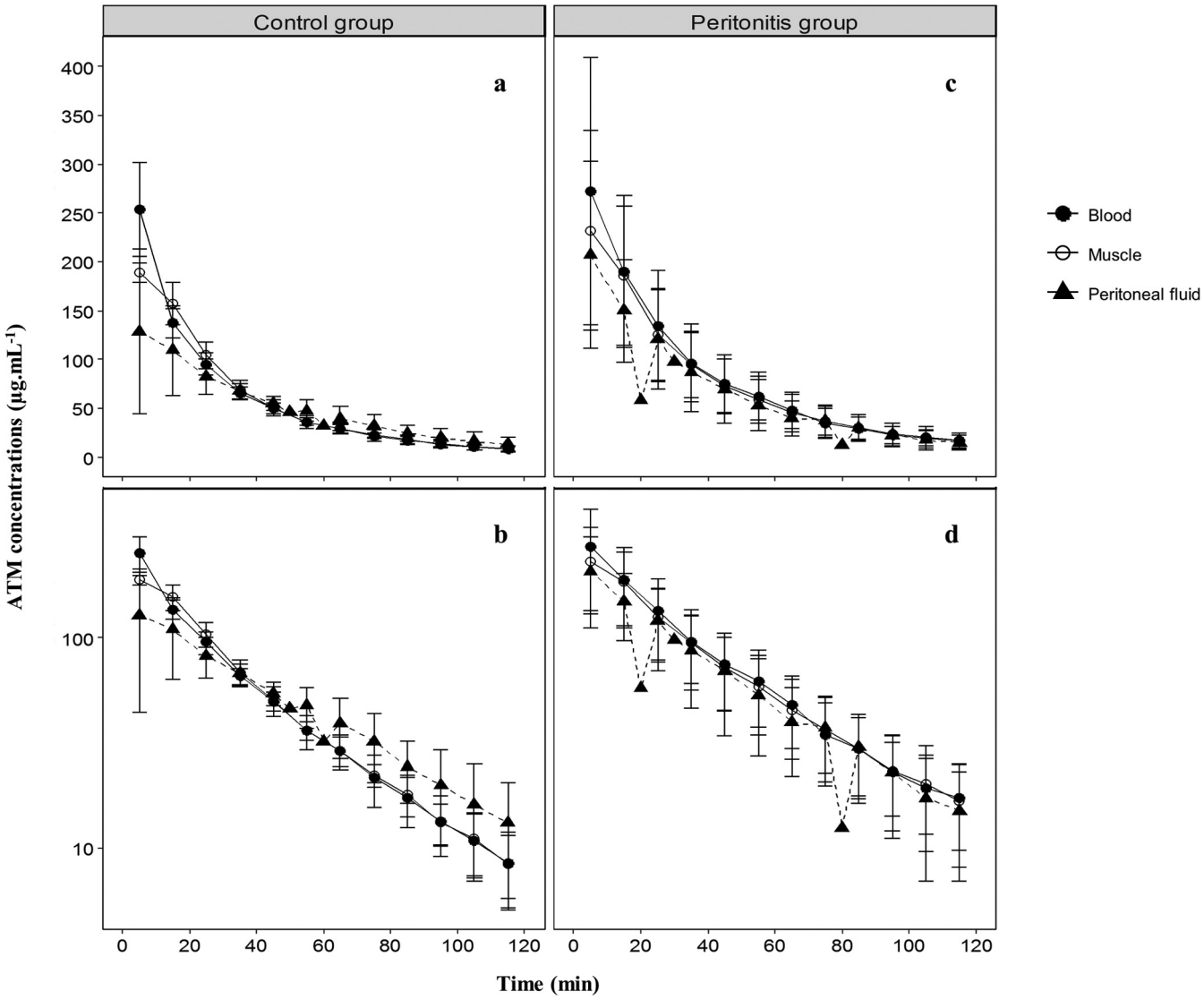

FIG 1 Unbound ATM concentrations in blood, muscle, and PF after an i.v. bolus of the ATM-AVI combination at a dose of 100-25 mg · kg^{-1}. Mean ATM concentrations ± the SD in control rats (a, decimal scale; b, semilogarithmic scale) and in rats with peritonitis (c, decimal scale; d, semilogarithmic scale) are depicted.

Numerous microdialysis studies have been conducted in rats to investigate antibiotics distribution in tissues (8) but not for ATM and AVI. This approach allows determination of unbound drug concentrations continuously in different tissues, but in the present study microdialysis was also used to determine unbound drug concentrations in blood by introducing a microdialysis probe in a vein, as previously described (13, 14). Unbound blood concentrations thus determined were compared to unbound concentrations measured in tissues for the same rat without performing repeated blood sampling, which may induce hypovolemia and therefore alter drug distribution characteristics (15). Probe recoveries varied widely between rats and between media (blood, muscle, and PF), but this variability was comparable to that previously observed for other β-lactams, such as imipenem, in the same tissues (16–18). Probe recoveries were generally higher for AVI than for ATM, possibly due to the fact that AVI has a lower molecular weight than ATM (435.43 g · mol^{-1} for ATM and 265.24 g · mol^{-1} for AVI), considering that both molecules present similar characteristics in terms of hydrophilicity (LogD at pH 7.4 = −6.02 for ATM and −6.77 for AVI) (ChemSpider).

ATM and AVI pharmacokinetic parameters were derived from unbound blood

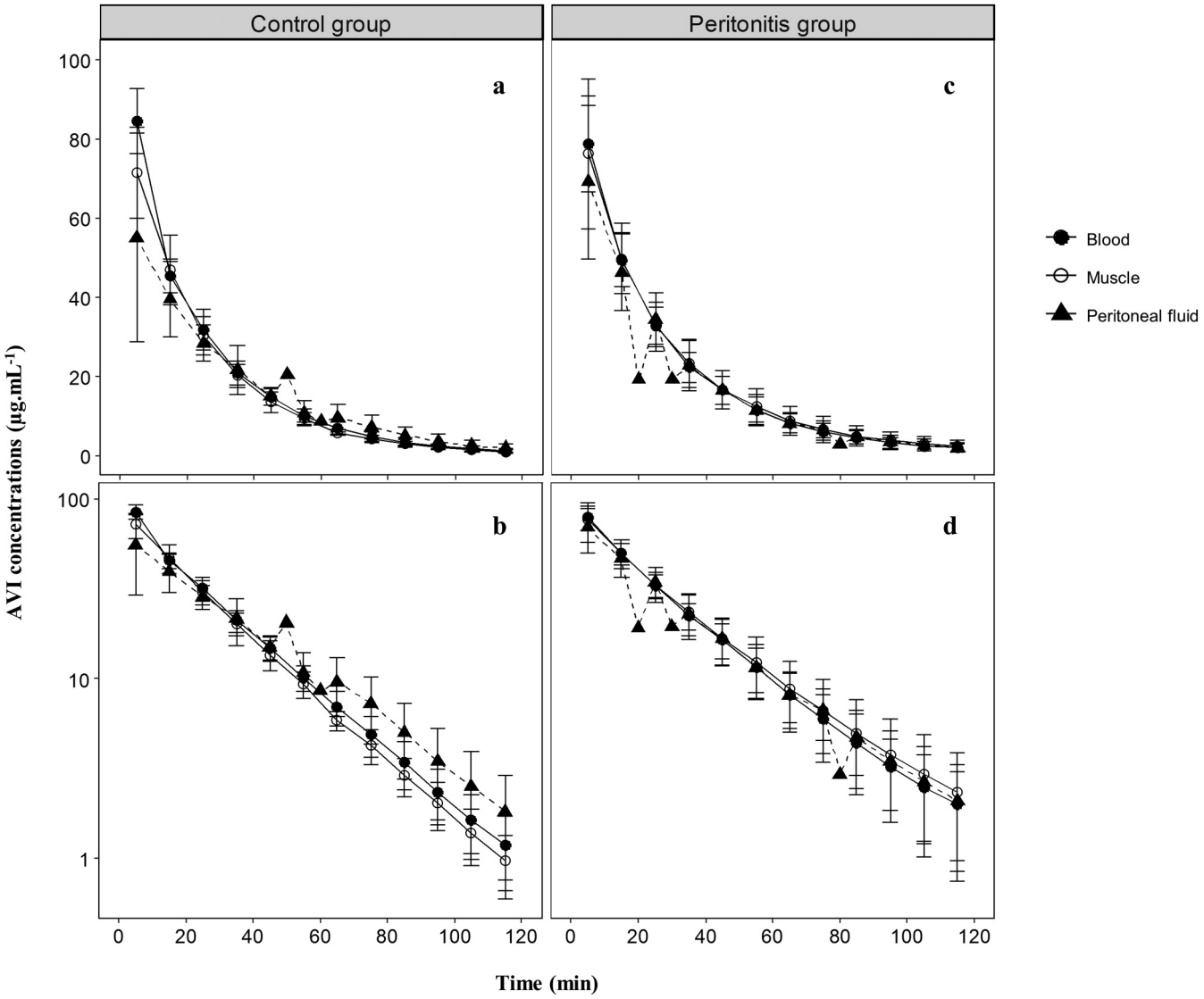

FIG 2 Unbound AVI concentrations in blood, muscle, and PF after an i.v. bolus of the ATM-AVI combination at a dose of 100-25 mg · kg^{-1}. Mean AVI concentrations ± the SD in control rats (a, decimal scale; b, semilogarithmic scale) and in rats with peritonitis (c, decimal scale; d, semilogarithmic scale) are depicted.

concentrations and therefore could not be compared, except for the half-life, with previously reported values determined using total concentrations. The mean ATM half-life determined in control rats (25.8 min) was consistent with the previously obtained value (24.6 min) (19), whereas regarding AVI, a study performed in rats with intra-abdominal abscesses reported a higher half-life value (43.8 min) (20) than those determined in the present study (18.8 min in control rats and 21.4 min in rats with peritonitis). ATM and AVI are mainly excreted unchanged in urine; however, in the present study, mean unbound clearances of ATM and AVI in control rats (12.8 ml · min^{-1} · kg^{-1} and 10.6 ml · min^{-1} · kg^{-1}, respectively) were greater than the glomerular filtration rate in rats (5.24 ml · min^{-1} · kg^{-1}) (21), suggesting that these compounds are not only filtered through the kidney but also actively secreted (22, 23). The mean ATM volume of distribution (473 ml · kg^{-1}), unlike that for AVI (285 ml · kg^{-1}), was slightly higher than the total extracellular body water volume (297 ml · kg^{-1}) (21). The first main observation, in the present study, was that ATM and AVI AUCs were not statistically different between blood, muscle, and PF in control group, which is consistent with basic pharmacokinetic concepts (24) and with previous results observed in tissues

TABLE 1 ATM unbound pharmacokinetic parameters values (mean ± SD) estimated in blood, muscle, and PF of control rats and rats with peritonitis after an i.v. bolus of ATM-AVI combination at a dose of 100-25 mg · kg^{-1}

	Control rats			Rats with peritonitis		
Parameter	Blood	Muscle	Intraperitoneal fluid	Blood	Muscle	Intraperitoneal fluid
V_u (ml · kg^{-1})	473 ± 75			503 ± 328		
CL_u (ml · min^{-1} · kg^{-1})	12.8 ± 1.8			11.7 ± 7.9		
$t_{1/2}$ (min)	25.8 ± 4.7	26.4 ± 7.1	33.2 ± 10.3	30.2 ± 2.9	30.8 ± 5.6	31.0 ± 4.1
AUC (μg · min · ml^{-1})	7,907 ± 1,008	7,421 ± 451	6,972 ± 1,120	10,834 ± 4,475	10,193 ± 3,866	9,037 ± 3,939
$AUC_{tissue}/AUC_{u,blood}$		0.95 ± 0.12	0.89 ± 0.14		1.00 ± 0.30	0.92 ± 0.41

without efflux transporter or peripheral degradation, such as lung and muscle (18, 25, 26). Moreover, the second main observation was that mean pharmacokinetic parameters of both molecules were not affected by the infection, even though between-rat variability was higher in infected animals, as it was also observed in humans with other antibiotics (27, 28). The lack of effect of peritonitis on ATM and AVI distribution in PF is consistent with what was previously observed in an intraperitoneal microdialysis study of imipenem in rat (16). However, this observation is not in agreement with results obtained during previous microdialysis studies conducted with carbapenems (imipenem and meropenem) (2, 29) in critical care patients with peritonitis where some intraperitoneal degradation was described for these antibiotics. Therefore, a clinical study with ATM and AVI in humans could be necessary to further investigate this issue even if the cecal ligation and puncture (CLP) model used in the present experiment is considered the gold-standard model for polymicrobial septic peritonitis (30, 31). This model that mimics the hemodynamic (increased blood flow in the early stage, followed by decreased blood flow in the late stage) and metabolic changes (hyperglycemia and hyperinsulinemia in the early phase, followed by hypoglycemia, hypoinsulinemia, and increased serum lactates during the late stage) of human disease (30, 32–34) can be easily reproduced in animals, as shown in the present study, where an increase in lactate concentrations and hypoglycemia were observed in the peritonitis group. However, a recent study compared the CLP model with the colon ascendens stent peritonitis (CASP), which is another model of polymicrobial sepsis where a stent is surgically inserted into the ascending colon and causes continuous influx of enteric bacteria into the peritoneal cavity, and showed that the two models could reflect different kind of diseases (35). The CASP model, resulting in a continuously increasing systemic infection and inflammation, resembles diffuse peritoneal infection, while CLP, producing more moderate inflammation and local bacterial spread, represents a model of intra-abdominal abscess formation. Therefore, since CLP and CASP result in different pathophysiological and clinical features, the choice of the animal model could have an impact on the pharmacokinetic and the distribution of ATM and AVI within tissues.

In conclusion, this study has clearly demonstrated that ATM and AVI distribute rapidly and freely in muscle and PF, both in the absence and in the presence of infection induced by cecal ligation and puncture in rats, suggesting that unbound ATM and AVI concentrations in blood should reflect unbound concentrations at the infection site and should therefore be used to predict the antibacterial effect.

TABLE 2 AVI unbound pharmacokinetic parameters values (mean ± SD) estimated in blood, muscle, and PF of control rats and rats with peritonitis after an i.v. bolus of ATM-AVI combination at a dose of 100-25 mg · kg^{-1}

	Control rats			Rats with peritonitis		
Parameter	Blood	Muscle	Intraperitoneal fluid	Blood	Muscle	Intraperitoneal fluid
V_u (ml · kg^{-1})	285 ± 43			312 ± 40		
CL_u (ml · min^{-1} · kg^{-1})	10.6 ± 1.0			10.2 ± 1.2		
$t_{1/2}$ (min)	18.8 ± 2.9	18.1 ± 2.9	21.4 ± 5.0	21.4 ± 3.0	22.4 ± 4.7	22.8 ± 4.4
AUC (μg · min · ml^{-1})	2,382 ± 210	2,154 ± 293	2,107 ± 374	2,486 ± 339	2,523 ± 496	2,328 ± 600
$AUC_{tissue}/AUC_{u,blood}$		0.91 ± 0.11	0.88 ± 0.11		1.01 ± 0.14	0.94 ± 0.21

MATERIALS AND METHODS

Chemicals. Aztreonam (Azactam; Sanofi-Aventis Laboratories, Paris, France) and avibactam (provided as a dry powder by Astrazeneca, Macclesfield, United Kingdom) were used to prepare ATM-AVI solutions in 0.9% NaCl or Ringer solution for intravenous (i.v.) administration and probe perfusion, respectively. All chemicals used were of analytical grade.

Animals. Experiments were carried out in accordance with EC Directive 2010/63/EU. They were approved by the local ethics committee (COMETHEA) and registered by the French Ministry of Higher Education and Research (authorization number 2016011111381822). Fourteen male Sprague-Dawley rats from Charles River Laboratories (Saint-Germain-Nuelles, France) weighing 291 ± 16 g (mean ± the standard deviation [SD]) were used and divided into two groups: a peritonitis group ($n = 9$) and a control group ($n = 5$). All animals were acclimatized in ventilated rack in temperature regulated environment with a 12-h light-dark cycle, with free access to food and water for a minimum of 5 days before the beginning of the experiment.

Surgery. (i) Catheter, vein and muscle probes insertion. The day before the experiment, rats received subcutaneous (s.c.) administration of buprenorphine at a dose of 0.05 mg · kg^{-1} before being anesthetized by isoflurane (Forene; Abbot, Rungis, France) inhalation (3% in inhalation chamber, followed by 1% under mask). A polyethylene cannula was then inserted into the left femoral vein for drug administration, as previously described (25). Two CMA/20 probes (polycarbonate; cutoff, 20,000 Da; membrane length, 10 mm; CMA microdialysis; Phymep, Paris, France) were inserted into the right jugular vein and the right hind leg muscle, as previously described too (25). In brief, the microdialysis CMA/20 probe, perfused with 1% low-molecular-weight heparin at a flow rate of 2 μl · min^{-1} (CMA 100 microdialysis pump; Phymep), was inserted through the pectoral muscle into the right jugular vein with the help of an introducer (needle inserted into a tube) and then secured by suturing it to the pectoral muscle. The probe, perfused with Ringer solution (perfusion fluid T1 for peripheral tissue; CMA microdialysis; Phymep) was inserted into the right hind leg muscle as previously described and finally secured by suturing it to the muscle. After insertion, the microdialysis probes were flushed at 10 μl · min^{-1} for approximately 15 min to remove bubbles. The flow rate was then decreased to 2 μl · min^{-1} until the end of the surgery. The inlet and the outlet of probes as the femoral catheters were then passed subcutaneously to exit at the nape.

(ii) Induction of peritonitis. Directly after installation of vein catheter and muscle microdialysis probe, peritonitis was induced in rats of the infected group, as previously described (31, 32). Briefly, after a laparotomy, the cecum was ligated just below the ileocecal valve, punctured three times below the ligature, and kneaded to get the contents out before being placed back into the peritoneal cavity. The abdomen was closed and, at the end of surgery, the rats were allowed to recover consciousness. Food was withdrawn approximately 12 h before the experiment, but the animals had free access to water and, at this time, they received a second s.c. injection of buprenorphine at the same dose as previously.

(iii) Intraperitoneal probe implantation. On the day of the experiment, the rats were reanesthetized by isoflurane (Forene; Abbot) inhalation. Another CMA/20 probe, perfused with Ringer solution (perfusion fluid T1 for peripheral tissue; CMA microdialysis; Phymep), was inserted into the rat peritoneal cavity between intestinal loops through laparotomy, as previously described (16). After insertion, the intraperitoneal microdialysis probe was flushed as previously done for the muscle probe. The probe was then sutured to the abdominal muscle, and the abdominal cavity was closed.

Pharmacokinetic study. (i) Recovery calculations. The pharmacokinetic experiment started with a retrodialysis by drug period, during which probes were perfused for 15 min at 2 μl · min^{-1} and then for 45 min at 1 μl · min^{-1} with Ringer containing ATM-AVI (10 and 10 μg · ml^{-1}) to equilibrate the system. After this equilibration period, microdialysate samples were collected for 1 h by fractions corresponding to 20-min intervals. To determine the *in vivo* recovery by loss ($RL_{in\ vivo}$), the ATM and AVI concentrations in the perfusate (C_{in}) and in dialysates (C_{out}) were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The $RL_{in\ vivo}$ was expressed as a percentage and was calculated for each interval of time as follows: $RL_{in\ vivo} = [(C_{in} - C_{out})/C_{in}] \times 100$. The *in vivo* recovery used to correct the dialysate concentrations was the mean value obtained from the three individual determinations. A washout period of 1 h (15 min at 2 μl · min^{-1} and 45 min at 1 μl · min^{-1}) with blank Ringer solution perfusion was allowed before i.v. ATM-AVI administration to remove the ATM and AVI from the probes. The flow rate was maintained at 1 μl · min^{-1} for the rest of the study.

(ii) ATM-AVI administration. The aztreonam-avibactam combination was administered as an i.v. bolus at a dose of 100-25 mg · kg^{-1}.

(iii) Microdialysis experiment. In each group, 10 min after the beginning of the i.v. ATM-AVI administration (the time needed to flush the dead volume), the dialysates in muscle, peritoneal fluid (PF), and blood were collected automatically by a Univentor/820 microfraction collector (Phymep) over 120 min at 10-min intervals. At the end of the experiment, two blood samples were collected via an intracardiac puncture, one in a tube containing fluoride and one in a heparinized tube, and then centrifuged at 1,000 × *g* for 10 min at 4°C. The supernatants were used for lactate and glucose determinations.

Microdialysis sample analysis. Analysis of ATM and AVI in the dialysates was performed by an adaptation of two separate LC-MS/MS methods, previously described and developed for the quantification of ceftazidime and avibactam in human plasma samples (36). Calibration curves were established in Ringer solution over 0.05 to 10 μg · ml^{-1} (AVI) and 0.1 to 200 μg · ml^{-1} (ATM). Directly after collection, microdialysates were diluted (1:3 [vol/vol]) with Ringer solution and were directly subjected to onto LC-MS/MS. The system included a Shimadzu high-performance liquid chromatography system module (Nexera XR; Shimadzu, Marne la Vallée, France) coupled with an API 3000 mass spectrometer (Sciex, Les

Ulis, France). ATM was analyzed on an XBridge C_{18} column (3.5 μm, 50 by 2.1 mm [inside diameter]; Waters, Saint-Quentin en Yvelines, France). The mobile phase A consisted of water with 0.05% trifluoroacetic acid, and mobile phase B was acetonitrile with 0.05% trifluoroacetic acid. The gradient for mobile phase B was set at 5, 90, and 5% at 0.01, 1, and 3 min, respectively, with a flow rate of 0.3 ml · min^{-1}. AVI was analyzed on an Acquity BEH amide column (1.7 μm, 50 by 2.1 mm [inside diameter]; Waters), and a mobile phase composed of 100 mM ammonium formate and acetonitrile (5:95 [vol/vol]) was delivered isocratically at 0.2 ml · min^{-1}. Electrospray ionization in negative mode was used for the detection of ATM and AVI. Ions were analyzed in the multiple reaction monitoring, and the following transitions were inspected: *m/z* 434.1→122 for ATM, *m/z* 440.1→122 for its deuterated internal standard, and *m/z* 264.1→96 for AVI and *m/z* 270.1→96 for the AVI labeled internal standard. The intraday variability was characterized at four concentration levels (200, 5, 0.2, and 0.1 μg · ml^{-1} for ATM; 10, 2, 0.1, and 0.05 μg · ml^{-1} for AVI) with a precision and bias of <10% for both compounds (n = 5 per molecule). Corresponding between-day variability was determined with a precision and a bias of <10% (n = 15).

Glucose and lactate determinations. Glucose and lactate concentrations were measured, respectively, by enzymatic and colorimetric methods in Cobas 8000 device (Roche Diagnostics, Meylan, France). Glucose levels could be determined in only 11 of 14 rats (2 control rats and 9 infected rats) due to technical issues.

Noncompartmental pharmacokinetic analysis. Pharmacokinetic parameters were determined in each individual rat by a noncompartmental approach according to standard procedures and with the software Phoenix WinNonLin 6.2 (Certara, Princeton, NJ). The total unbound body clearance (CL_u) was calculated as CL_u = dose/$AUC_{u,blood}$, where $AUC_{u,blood}$ is the total area under the unbound blood concentration-versus-time curve from zero to infinity, calculated by the trapezoidal rule. The area remaining under the curve after the last measured concentration, $C(last)_{u,blood}$, was determined from $C(last)_{u,blood}/k_{el,blood}$. The elimination rate constant ($k_{el,blood}$) and its corresponding half-life ($t_{1/2,blood}$) were estimated by a least-squares fit of the data points (log concentration-time) in the terminal phase of the decline. The volume of distribution (V_u) was obtained from $CL_u/k_{el,blood}$. The AUC and $t_{1/2}$ in tissues were also estimated by the same procedure and are referred as AUC_{muscle}, AUC_{PF}, $t_{1/2,muscle}$, and $t_{1/2,PF}$.

Statistical analysis. Concentrations were expressed as means ± the standard deviations (SD). However, when probe recoveries were lower than 10% in some tissues, concentrations were excluded from the analysis since small recovery values result in large errors in the estimation of the concentrations. Comparisons of AUC and $t_{1/2}$ estimated in blood, muscle and PF were performed for each group of rats by a nonparametric Kruskal-Wallis test. The AUC, $t_{1/2}$, CL_u, V_u, $AUC_{muscle}/AUC_{u,blood}$ ratio, $AUC_{PF}/AUC_{u,blood}$ ratio, and lactate and glucose concentrations between the two groups were compared by a Mann-Whitney test. Significance was set at a P level of <0.05.

ACKNOWLEDGMENTS

This research project is partially funded by the Innovative Medicine Initiative Joint undertaking under COMBACTE-CARE grant agreement 115620, resources of which are composed of financial contributions from the European Union Seventh Framework Programme (FP7/2007-2013) and European Federation of Pharmaceutical Industries and Associations (EFPIA) companies in kind contribution. Avibactam dry powder was provided by AstraZeneca.

We thank program Nouvelle Aquitaine CPER 2015-2020 and FEDER 2014-2020 for their participation in equipment funding. The biochemistry department of Poitiers University Hospital is also acknowledged for its assistance in lactate and glucose determinations. This study has benefited from the facilities and expertise of PREBIOS platform (University of Poitiers).

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